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Understanding COVID-19; Are children the key?

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Understanding COVID-19; Are children the key?

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ABSTRACT

The devastating impact of the coronavirus 2019 (COVID-19) pandemic on global health and economic stability is immeasurable. The situation is dynamic and fast evolving with the world now facing variants of concern which may have immune escape potential. With threatened treatment and preventative strategies at stake, and the prospect of reinfection prolonging the pandemic, it is more crucial than ever to understand the pathogenesis of SARS-CoV2 infection which intriguingly affects adults and the elderly more severely than children.

Children infected with SARS-CoV2 remain largely asymptomatic or undergo a transient mild illness. Understanding why children experience a milder phenotype and a significant survival advantage may help identify modifiable risk factors in adults.

Current evidence suggests adults with COVID-19 show variability in innate and adaptive immune responses, which may result in uncontrolled pro-inflammatory cytokine production in some patients, leading to severe disease and mortality. Children with acute COVID-19 infection seldom progress to ARDS and are less likely to exhibit the cytokine storm which is so prominent in adults. Even with the Kawasaki like illness, a hyperinflammation syndrome also known as paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV2 (PIMS-TS), mortality is low.

The key to successfully combating SARS-CoV2 and future zoonotic pandemics may lie in understanding these critical differences and merits focussed consideration and research. The impact of community transmission amongst asymptomatic children is unknown; vaccination programmes should include children who are potential reservoirs of infection spread. We discuss the fundamental differences in the

immune response, ACE2 expression and transmission between children and adults in the fight against SARS-CoV2.

Key Statements:
Significant differences in immune response to SARS-CoV2 exists between children and adults; understanding the basis for this variance may be crucial to controlling the pandemic.
The role of children in the transmission of emerging SARS-CoV2 variants, which are more contagious and possibly more virulent in adults, needs to be established.
The pandemic will not be controlled without global COVID-19 vaccination which must include vaccinating children who are potential reservoirs of transmission and infection.

INTRODUCTION

The coronavirus 2019 (COVID-19) pandemic has had a catastrophic impact on world health and economic stability. It is hoped that social normalcy and economic restoration will return with the success of the SARS-CoV2 vaccination programmes. The emergence of mutant strains raises concerns over the efficacy of recently licenced vaccines, questioning the degree of cross-immunity and protection afforded by these vaccines. Whether the programmes are successful or not, we must continue to decipher and understand the pathogenesis of this devastating infection which disproportionately affects adults and the elderly.

Children infected with SARS-CoV2 are either asymptomatic or experience a milder phenotype compared to adults [1-3]. This trajectory is similar to that experienced in the SARS-CoV1 and MERS epidemics of 2002 and 2012 respectively which differs from the common childhood respiratory infections such as RSV and *Mycoplasma pneumoniae* that are associated with greater morbidity [3]. In addition, COVID-19 symptoms of fever, cough and a loss of sense of smell or taste are less frequently encountered in children [1-3].

Pneumonitis progressing to ARDS and respiratory failure occurs in 14-19.5% of adult COVID-19 patients with case fatality rates (CFR) of 5-8%, rising to 14.8% in elderly patients >80yrs [4,5]. The prevalence of SARS-CoV2 in children has not been accurately determined. Children are quoted to account for 1- 5% of positive SARS-CoV2 PCR tests but this is likely to be underrepresented as testing is skewed towards those who are symptomatic or require hospitalisation [6,7]. Of the children who test positive, severe COVID-19 occurs in 1-6%, with 65% of these developing the Kawasaki-like illness, paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV2 (PIMS-TS), also known as multisystem inflammatory syndrome in children (MIS-C), but even then, mortality is low [8].

Despite the milder phenotype, the pandemic has had a detrimental effect on child and adolescent health and education worldwide [9]. Healthcare access and delivery are also severely disrupted with essential services like routine immunisations being

postponed [9-10]. Children are also considered at low risk from COVID-19 and are therefore not a priority in vaccination trials.

We review the differences and similarities in COVID-19 infection between children and adults, focussing on the immune response, its role in pathogenesis and the importance of childhood vaccination.

CHILDHOOD AND ADULT IMMUNE RESPONSES TO SARS-CoV2

The immune response in adults with severe COVID-19 is very different to children with acute SARS-CoV2 infection (Table 1). Adults with severe and critical COVID-19 display a dysregulated adaptive arm in combination with a hyperactivated innate response which leads to uncontrolled pro-inflammatory cytokine production, resulting in extensive immune-mediated lung injury and multi-organ failure [11,12]. Children with acute COVID-19 rarely develop pneumonitis and seldom manifest the hyperinflammation [11,12]. This cytokine storm however is part of the immunophenotype seen in PIMS-TS, a condition which characteristically occurs in children who are SARS-CoV2 PCR negative and seropositive [13, 14].

1. Innate Immunity

Hyperacute but ineffective innate immunity has been strongly associated with a lack of control of primary SARS-CoV2 infection and a high risk of fatal COVID-19 [11,12].

Circulating monocytes and neutrophils have a positive association with COVID-19 disease severity in adult studies. Fluorescence-activated cell sorting analysis (FACS) shows classical (CD14+ CD16-) and intermediate monocytes (CD14++ CD16+) are responsible for the production of the inflammatory cytokines seen in severe COVID-19 [11]. These activated innate cells are rarely found in asymptomatic individuals or those with mild disease. Interestingly, non-classical monocytes (CD14+ CD16++), which are anti-inflammatory and has a role in maintaining endothelial integrity, are reduced in severe COVID-19. [11,12].

Activated neutrophils produce neutrophil extracellular traps (NETS) which are aptly named net-like structures made up mostly of chromatin and extracellular proteins that 'trap' microbes and infected cells. These NETS can prime the clotting cascade. NETS are thus implicated in the microangiopathy and thrombosis seen in COVID-19 [12]. Children with PIMS-TS have been demonstrated to have endothelial cell damage and thrombosis, especially seen in their skin lesions [13]. The underlying microvascular and thrombotic pathogenesis may be shared in acute COVID-19 and PIMS-TS.

NK cells although are of lymphocyte lineage, possess fast acting innate properties of perforin and granzyme degranulation and are powerful killers of virus infected cells. NK cells are consistently reduced in COVID-19 but this is most pronounced in severe disease [11]. Lower numbers of perforin producing NK cells were found in intensive care unit (ICU) COVID-19 patients compared to non-ICU patients in one study [15]. NK cell numbers are preserved in children with non-severe COVID-19. FACS analysis of peripheral blood in patients with PIMS-TS however, revealed similar reductions in NK cells and non-classical monocytes to adults with severe COVID-19 [16].

Classical antigen presenting cells like dendritic cells (DCs) are also suppressed. DCs produce both interferon (IFN) I and III which are powerful anti-viral cytokines important in early viral responses. SARS-CoV2 like SARS-CoV and MERS, possess this immune escape ability of IFN inhibition [12,17]. Reduced antigen presentation by DCs downregulates T cell activation, affecting T cell function and expansion [12].

Another theory for why children are more resilient to acute COVID-19 is trained immunity. Trained immunity is the functional reprogramming of innate immune cells following their epigenetic modification from vaccinations and common childhood infections, thus enabling memory cell formation which was previously considered unique to the adaptive immune system [1,3,7]. Trained immunity is described with the Bacillus Calmette–Guérin (BCG) vaccination, which affords heterogeneous immunity against other pathogens [18,19]. Trained immunity may also be derived

from co-infections; 10% of co-infections occur in children testing positive for SARS-CoV2 [7,19].

2. Adaptive immunity

a. Lymphopenia & lymphocyte exhaustion

The lymphopenia and high neutrophil-to-lymphocyte ratio described in hospitalised adult patients with acute COVID-19 is rarely observed in children [20,21]. In adults, CD4+ and CD8+ T cells and both naïve and memory T cell numbers are reduced [22]. Conversely, lymphocyte counts are preserved in children with acute COVID-19 but reduced in PIMS-TS [7,16].

Adult patients with mild COVID-19 were found to have clonally expanded CD8+ T lymphocytes [22]. Moreover, individuals with mild disease were shown to mount a stronger SARS-CoV2 specific CD4+ and CD8+ T cell response compared to those with severe disease implying preserved adaptive response corresponds with superior disease control and recovery [12,22]. Preservation of T cell numbers and their subsets, helper T cells, anti-viral cytotoxic T cells and a functional homeostatic immune arm with sustained Treg capability may be protective and account for higher viral clearance in children.

In addition to lymphopenia, T cell exhaustion is also reported in adults.
T lymphocytes from severe COVID-19 patients have higher expression of the cell exhaustion and inhibition markers PD-1, LAG-3 and TIM3 [21]. Compared to healthy controls, CD8+ T lymphocytes and NK cells in COVID-19 patients also have a higher surface expression of the NKG2A inhibitory receptor; levels return to normal with COVID-19 recovery [12,21]. T cell exhaustion correlates with impaired effector function. These findings suggest that cellular immunity is affected both quantitatively and functionally. T cell function and survival is a potential novel area of research in children with COVID-19; findings will contribute to our understanding of the age-related T cell response and behaviour in COVID-19.

b. Anamnestic responses

The course of severe COVID-19 in adults is relatively slow (median 19 days post-symptom onset for fatal cases [23,24]), which suggests the protective involvement of memory B cells and T cells that take days to develop [25]. The Crotty group reported that virus-specific memory CD4+ and CD8+ T cell responses declined quickly in adults with a half-life of 3.5 months, whereas virus-specific B cell responses were more abundant at 6 months compared to 1 month after infection [26]. Moreover, SARS-CoV-2-specific memory B cell responses evolved between 1.3 and 6.2 months after infection in a manner that was consistent with antigen persistence; viral antigens were detectable in intestinal biopsies from adults even months after infection [27].

Immune responses shortly after resolution of infection are not predictive of long-term memory in adults [28]. Learning from SARS-CoV-1 and MERS, it is likely that antibodies persist for 2-3 years after infection but T cell memory responses may last longer. Indeed, 28-50% of adults not previously exposed to SARS-CoV-2 infection had cross-reactive T cells [29] likely mounted against other members of the coronavirus family that includes the common cold coronaviruses HCoV-OC43, HCoV-HKU1, HCoV-229E and HCoV-NL63. A similar analysis would be particularly interesting in children, to establish if common cold-associated coronavirus infections add to their defences against COVID-19.

A study in hospitalised adults and children with COVID-19 in New York added to the cumulative evidence that the milder disease seen in children was unlikely owed to adaptive immune responses; adults with severe COVID-19 mounted strong T cell responses and had higher neutralizing antibodies compared to children with COVID-19 and/or PIMS-TS [24].

c. Humoral response

COVID-19 patients mount a strong B cell response; neutralizing antibodies against the Spike glycoprotein have been detected in the majority of convalescent COVID-19 patients [12].

Although total B cells numbers are reduced, plasma cell levels are paradoxically increased [11]. IgG and IgA levels are transiently increased in adult asymptomatic

patients which waned over time; this is observed to a lesser extent in those with mild disease [11]. Patients with severe COVID-19 on the other hand, have persistently raised IgG and IgA levels. IgM levels were equally raised in the early phase in asymptomatic, mild and severe cases [11]. Children with non-severe COVID-19 were found to have early phase rise in IgM, persistent IgG levels and waning IgA levels in the convalescent phase [11,12]. In contrast, PIMS-TS patients were shown to mount a robust antibody response with high IgG and IgA titres against the viral Spike glycoprotein [14]. Notably, no IgM was detected in these children who were also SARS-CoV2 PCR negative, signifying that PIMS-TS is a clinical syndrome which occurs weeks following the acute infection [14].

Children are also speculated to be afforded additional cross-protective antibodies against SARs-CoV2 from seasonal coronaviruses which commonly infect children [1-3,7].

Declining immune cell function, termed immunosenescence, naturally occurs with age and is most pronounced in elderly patients >80yrs old. Dampened adaptive immunity and defective innate immune responses are observed [1,3,7,12]. Immunosenescence may therefore predispose older patients to poor viral clearance and promote COVID-19 disease progression.

3. **Cytokine storm**

A hyperinflammatory response, referred to as the 'cytokine storm' occurs in the second week in adults with severe and life-threatening COVID-19. Higher circulating pro-inflammatory cytokines are observed and clinically patients develop ARDS, multiorgan failure and DIC [4,5]. The cytokine storm is preceded by pulmonary infiltration of macrophages and neutrophils. These innate cells secrete powerful inflammatory cytokines including interleukin- 6 (IL-6), IL-12, IL-10, IFN-γ and TNF-α and with varying reports of raised IL-1β, IL-7, IL-8, IL-17 and G-CSF [19,30]. Levels of IL-6, IL-10 along with C-reactive protein (CRP) are considered prognostic markers [19,30]. Lymphopenia, raised ferritin and D-Dimer levels also correspond with disease severity in adult COVID-19 patients [5].

Although children rarely manifest the cytokine storm with acute COVID-19 infection, the macrophage activation syndrome (MAS) and subsequent systemic inflammatory response is seen in PIMS-TS [14,24,31]. MAS is encountered infrequently with other childhood infections e.g. Epstein-Barr virus (EBV). PIMS-TS presents with a wide array of clinical features and variable disease severity with shock, multi-organ failure, left ventricular impairment and coronary artery abnormalities described amongst them [31].

Lower levels of proinflammatory cytokines and CRP are reported in children with acute COVID-19 [30]. Several studies found levels of IL-6, IFN-γ and TNF-α to be unchanged [20]. Pierce and colleagues however, found children with PIMS-TS to have higher levels of IL-6, IFN-γ, TNF-α and CXCL10 compared to children with acute COVID-19 [24]. The chemokines CXCL10 (aka IP-10), CXCL8, CCL2 (aka MCP-1) are likewise detected in large amounts in adult COVID-19 [30].

In terms of laboratory biomarkers, PIMS-TS patients, similar to severe adult COVID-19, have higher CRP, ferritin and D-Dimer levels, reflecting the inflammatory nature of the underlying pathology [32]. The similarity in their immunophenotype and biomarker levels suggests the underlying immunopathogenesis is the same, but it is the end organ response that is different between severe adult COVID-19 and PIMS-TS. Based on our experience at Birmingham Children's Hospital, UK, PIMS-TS does not appear to be a direct result of SARS-CoV2 infection but occurs following host immune attenuation after SARS-CoV2 exposure [14]. This concept is supported by the observation that PIMS-TS cases arose weeks following the peak of the 1st wave of COVID-19 and most children were SARS-CoV2 PCR negative and IgG positive at the time of presentation [14,31].

4. ACE2 expression

Children have a high expression of the SARS-CoV2 entry receptor, Angiotensin-converting enzyme 2 (ACE2) which although increases with age, starts to decline in old age [1,3,6]. ACE2 is found in most tissues but are highly expressed on type 2

pneumocytes. ACE2 downregulation is also seen in adults with comorbidities such as hypertension and diabetes and also upon SARS-CoV2 Spike protein binding [1,3]. Animal studies have shown ACE2 protects against lung injury induced by SARS-CoV2 [3, 6]. Paradoxically, lower TMPRSS2 levels are found in children and infants. TMPRSS2, transmembrane serine protease 2, helps to prime the Spike glycoprotein and promotes viral entry. [33]

These differences in ACE2 and TMPRSS2 expression may be pivotal in helping us understand the discrepancies in the clinical outcomes between children and adults. Lastly, despite their higher expression, ACE2 in children are speculated to have a lower affinity to SARS-CoV2 thus preventing viral entry into the host cell [6]. The affinity level may change with emerging SARS-CoV2 variants and it is possible that children may be more vulnerable to disease with these new strains.

TRANSMISSION

SARS-CoV2 transmits by aerosol and droplets but the virus is also detected in stool samples [34]. Children tend to experience more gastrointestinal symptoms of diarrhoea and vomiting than to adults, however, faecal-oral transmission is not a common route of infection spread [34].

The role for children in spreading the virus is considered to be small, and reflective of levels of transmission in their community. The potential to transmit infection, however, increases with age [35] and with different coronavirus variants. The UK government's New and Emerging Respiratory Virus Threats advisory group (Nervtag) reported increased concern that the B.1.1.7 variant, aka N501Y, which is 50-70% more transmissible in adults, may also be more easily transmissible in children. This is because the virus is suspected to require lower amounts of the ACE2 receptor for infection (https://www.bbc.co.uk/news/uk-55406939).

School and childcare centre COVID-19 outbreaks are not common especially if hand hygiene, wearing face masks (amongst teaching staff) and social distancing rules are followed [34], yet in the absence of testing asymptomatic individuals we are unable to track virus circulation in these settings. To date, contact tracing studies

show 71-90% of childhood SARS-CoV2 infections are usually identified following infection by a family member [36], so we are probably underestimating the proportion of asymptomatic infected children.

VIROLOGY AND VACCINES

The recent surge in COVID-19 numbers in the UK is attributed to the mutant SARS-CoV2 strain N501Y [37]. Another new variant, the E484K mutation which was first detected in South Africa, is now spreading rapidly throughout South Africa and a number of nations including Brazil and the UK [38]. Worryingly these new strains are not only more infectious but are more virulent, causing even greater morbidity and mortality [37,38]. There is no evidence to-date to establish children's susceptibility to the new variants, yet school closures in January 2021 in the UK were implemented to curb transmission of the more infectious strains.

With the SARS-CoV2 vaccination programmes underway, there are serious concerns that mutations in the Spike glycoprotein may render vaccine-induced antibodies ineffective or subneutralising [39]. The current COVID-19 vaccines are still considered to be effective against the N501Y variant but may be less so against E484K [38, 39].

The UK has approved vaccines by Oxford/AZ, Pfizer/BioNTech and Moderna, which are based on adenovirus vector (Oxford/AZ) or mRNA technologies. Of these, only the Pfizer BioNTech has been tested in children older than 12yrs of age.

Data from the Phase 3 ENSEMBLE trial of the new adenovirus vector vaccine by Janssen Pharmaceuticals has shown high titres of neutralising antibodies following a single dose thus eliminating the need for a booster dose (https://www.nih.gov/janssen-investigational-covid-19-vaccine-interim-analysis-phase-3). The Janssen vaccine and Novavax, a protein adjuvant vaccine, are pending approval, and have not been tested in children.

Due to the global push to prevent mortality in high-risk groups, children are a low priority group for vaccination. Currently, in the 1st phase of the UK vaccination programme, only children aged 12yrs and older with severe neurological disabilities and recurrent respiratory tract infections who require residential care, and young people aged 16yrs and older with specific clinical vulnerabilities will be considered for vaccination (https://www.rcpch.ac.uk/news-events/news/message-paediatricians-children-young-people-covid-19-vaccination-programme).

However, well children are potential reservoirs of transmission and infection and are thus an important group to vaccinate. The United States saw a large reduction in rates of invasive pneumococcal disease in all ages caused by serotypes included in the pneumococcal vaccines PCV7 and PCV13 after routine use of these vaccines began for children (https://www.cdc.gov/vaccines/vpd/pneumo/public/index.html). Global decline in infection rates and control of the COVID-19 pandemic may not occur until children are vaccinated too.

SUMMARY

Striking clinical and immune differences exist between children and adult COVID-19 responses. Children with acute COVID-19 infection are largely asymptomatic and rarely exhibit the dysregulated innate response seen in adults with severe disease. Adult severe COVID-19 and PIMS-TS likely share the same immunopathogenesis as demonstrated by their similar immunophenotypes yet their clinical response, time of onset from acute infection and end organ damage are distinctly different.

Due to their low risk of severe illness, children have not been a priority for vaccination, but this may be the key to controlling the pandemic as asymptomatic children are potential reservoirs of transmission and infection.

CONCLUSION

The COVID-19 pandemic has highlighted gaps in our knowledge of viral immunology between adults and children. Deciphering the mechanism of children's resistance to

disease could help target therapeutic interventions in adults and should be of high priority for future investigations.

Table 1. Differences in the immune response to COVID-19 in children and adults.

	ADULTS			CHILDREN			
	Severe	**Mild**	**Asymptomatic**	**PIMS-TS**	**Severe**	**Mild**	**Asymptomatic**
Innate Immunity	↑Monocytes	↑ Monocytes	↔Monocytes	↑Monocytes	n/a	↔Monocytes	↔ NK cells
	↓ NK cells	↓ NK cells	↑ NK cells	↓ NK cells	n/a	↑ NK cells	↑ NK cells
	↑ Neutrophils	↑ Neutrophils	↔ Neutrophils	↑ Neutrophils	n/a	↔ Neutrophils	↔ Neutrophils
Adaptive Immunity	Persistently ↑ IgG/IgA	↑ IgG/IgA	Transient IgG/IgA	Persistently ↑ IgG/IgA	Persistent ↑ IgG Transient ↑ IgA	Persistent ↑ IgG Transient ↑ IgA	Transient IgG/IgA
	↓Total B cells ↑ Plasma cells	n/a	↔Total B ↔Plasma cells	n/a	n/a	↔Total B ↔Plasma cells	↔Total B ↔Plasma cells
	↓CD3+ Total T cells	↑CD3+ Total T cells	↔CD3+ Total T cells	↓CD3+ Total T cells	↑CD3+ Total T cells	↑CD3+ Total T cells	↔CD3+ Total T cells
	↓CD8+ ↓CD4+	↑CD8+ ↑CD4+	↔CD8+ ↔CD4+	n/a n/a	↑CD8+ ↑CD4+	↑CD8+ ↑CD4+	↔CD8+ ↔CD4+
	T cell expression; ↑PD1, ↑LAG3, ↑TIM3 & ↑NKG2A	n/a	n/a	n/a	n/a	n/a	n/a
Cytokine	↑IL-6	↑IL-6	↔IL-6	↑IL-6	↔IL-6	↔IL-6	↔IL-6
	↑ IL-12	↑ IL-12	↔IL-12	n/a	n/a	n/a	n/a
	↑ IL-10	↑IL-10	↑ IL-10	↑ IL-10	↑ IL-10	↑ IL-10	↑ IL-10
	↑IFN	↑ IFN	↔IFN	↑IFN	↑/↔IFN	↑/↔IFN	↔IFN
	↑ TNFα	↑ TNFα	↔ TNFα	↑ TNFα	↔ TNFα	↔ TNFα	↔ TNFα
	↑/↔ IL-1β	↑/↔ IL-1β	↔ IL-1β	n/a	↔ IL-1β	↔ IL-1β	↔ IL-1β
	↑/↔ IL-7	↑/↔ IL-7	↔ IL-7	n/a	↔ IL-7	↔ IL-7	↔ IL-7
	↑/↔ IL-8	↑/↔ IL-8	↔ IL-8	↑ IL-8	↑ IL-8	↔ IL-8	↔ IL-8
	↑/↔ IL-17	↑/↔ IL-17	↔ IL-17	↑ IL-17	↑/↔ IL-17	↑/↔ IL-17	↔ IL-17
	↑/↔ G-CSF	↑/↔ G-CSF	↔ G-CSF	n/a	n/a	n/a	n/a
Chemokine receptors	↑CXCL10	↑CXCL10	↔CXCL10	↑CXCL10	n/a	↔CXCL10	↔CXCL10
	↑CXCL8	↑CXCL8	↔CXCL8	n/a	n/a	n/a	n/a
	↑CCL2	↑CCL2	↔CCL2	↔CCL2	↔CCL2	↔CCL2	↔CCL2

n/a, not available; ↑, increased; ↓, decreased; ↔, unchanged, IgG, immunoglobulin G; IgA, immunoglobulin A; PD1, Programmed cell death protein 1; LAG3, Lymphocyte activation gene 3;
TIM-3, T-cell immunoglobulin and mucin-domain containing-3; NKG2A, Natural Killer cell inhibitory receptor; NK cells, Natural killer cells; IL-, interleukin; IFN, Interferon; TNFα, Tumour necrosis factor alpha; G-CSF, Granulocyte colony stimulating factor; CXCL-, chemokine (C-X-C motif) ligand; CCL-, CC chemokine ligand; PIMS-TS,

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REFERENCES

1. Miri SM, Noorbakhsh F, Mohebbi SR, Ghaemi A. Higher prevalence of asymptomatic or mild COVID-19 in children, claims and clues. J Med Virol. 2020 Nov;92(11):2257-2259. doi: 10.1002/jmv.26069. Epub 2020 Jun 9. PMID: 32470174; PMCID: PMC7283682.

2. Epidemiology Working Group for NCIP Epidemic Response, Chinese Center for Disease Control and Prevention. [The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China]. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2020;41(2):145-151.PubMedGoogle Scholar

3. Dhochak N, Singhal T, Kabra SK, Lodha R. Pathophysiology of COVID-19: Why Children Fare Better than Adults? Indian J Pediatr. 2020 Jul;87(7):537-546. doi: 10.1007/s12098-020-03322-y. Epub 2020 May 14. PMID: 32410003; PMCID: PMC7221011.

4. Zhu J, Ji P, Pang J, Zhong Z, Li H, He C, Zhang J, Zhao C. Clinical characteristics of 3062 COVID-19 patients: A meta-analysis. J Med Virol. 2020 Oct;92(10):1902-1914. doi: 10.1002/jmv.25884. Epub 2020 Jun 24. PMID: 32293716; PMCID: PMC7262119.

5. Nanda A, Vura NVRK, Gravenstein S. COVID-19 in older adults. Aging Clin Exp Res. 2020 Jul;32(7):1199-1202. doi: 10.1007/s40520-020-01581-5. Epub 2020 May 10. PMID: 32390064; PMCID: PMC7211267.

6. Zimmermann P, Curtis N. Arch Dis Child Epub ahead of print: [please include Day Month Year]. doi:10.1136/ archdischild-2020-320338

7. Felsenstein S, Hedrich CM. SARS-CoV-2 infections in children and young people. Clin Immunol. 2020 Nov;220:108588. doi: 10.1016/j.clim.2020.108588. Epub 2020 Sep 6. PMID: 32905851; PMCID: PMC7474910.

8. Yasuhara J, Kuno T, Takagi H, Sumitomo N. Clinical characteristics of COVID-19 in children: A systematic review. Pediatr Pulmonol. 2020 Oct;55(10):2565-2575. doi: 10.1002/ppul.24991. Epub 2020 Aug 4. PMID: 32725955.

9. Araújo LA, Veloso CF, Souza MC, Azevedo JMC, Tarro G. The potential impact of the COVID-19 pandemic on child growth and development: a systematic review [published online ahead of print, 2020 Sep 23]. *J Pediatr (Rio J)*. 2020;S0021-7557(20)30209-6. doi:10.1016/j.jped.2020.08.008

10. Ashikkali L, Carroll W, Johnson C. The indirect impact of COVID-19 on child health. *Paediatr Child Health (Oxford)*. 2020;30(12):430-437. doi:10.1016/j.paed.2020.09.004

11. Carsetti R, Zaffina S, Piano Mortari E, Terreri S, Corrente F, Capponi C, Palomba P, Mirabella M, Cascioli S, Palange P, Cuccaro I, Milito C, Zumla A, Maeurer M, Camisa V, Vinci MR, Santoro A, Cimini E, Marchioni L, Nicastri E, Palmieri F, Agrati C, Ippolito G, Porzio O, Concato C, Onetti Muda A, Raponi M, Quintarelli C, Quinti I, Locatelli F. Different Innate and Adaptive Immune Responses to SARS-CoV-2 Infection of Asymptomatic, Mild, and Severe Cases. Front Immunol. 2020 Dec 16;11:610300. doi: 10.3389/fimmu.2020.610300. PMID: 33391280; PMCID: PMC7772470.

12. Bordallo B, Bellas M, Cortez AF, Vieira M, Pinheiro M. Severe COVID-19: what have we learned with the immunopathogenesis? Adv Rheumatol. 2020 Sep 22;60(1):50. doi: 10.1186/s42358-020-00151-7. PMID: 32962761; PMCID: PMC7506814.

13. Gkoutzourelas A, Bogdanos DP, Sakkas LI. Kawasaki Disease and COVID-19. *Mediterr J Rheumatol*. 2020;31(Suppl 2):268-274. Published 2020 Sep 21. doi:10.31138/mjr.31.3.268

14. Perez-Toledo M, Faustini SE, Jossi SE, Shields AM, Kanthimathinathan HK, Allen JD, Watanabe Y, Goodall M, Wraith DC, Veenith TV, Drayson MT, Jyothish D, Al-Abadi E, Chikermane A, Welch SB, Masilamani K, Hackett S, Crispin M, Scholefield BR, Cunningham AF, Richter AG. Serology confirms SARS-CoV-2 infection in PCR-negative children presenting with Paediatric Inflammatory Multi-System Syndrome. medRxiv [Preprint]. 2020 Jun 7:2020.06.05.20123117. doi: 10.1101/2020.06.05.20123117. PMID: 32577677; PMCID: PMC7302282.

15. Bordoni V, Sacchi A, Cimini E, Notari S, Grassi G, Tartaglia E, Casetti R, Giancola ML, Bevilacqua N, Maeurer M, Zumla A, Locatelli F, De Benedetti F, Palmieri F, Marchioni L, Capobianchi MR, D'Offizi G, Petrosillo N, Antinori A, Nicastri E, Ippolito G, Agrati C. An Inflammatory Profile Correlates With Decreased Frequency of Cytotoxic Cells in Coronavirus Disease 2019. Clin Infect Dis. 2020 Nov 19;71(16):2272-2275. doi: 10.1093/cid/ciaa577. PMID: 32407466; PMCID: PMC7239202.

16. Gruber CN, Patel RS, Trachtman R, Lepow L, Amanat F, Krammer F, Wilson KM, Onel K, Geanon D, Tuballes K, Patel M, Mouskas K, O'Donnell T, Merritt E, Simons NW, Barcessat V, Del Valle DM, Udondem S, Kang G, Gangadharan S, Ofori-Amanfo G, Laserson U, Rahman A, Kim-Schulze S, Charney AW, Gnjatic S, Gelb BD, Merad M, Bogunovic D. Mapping Systemic Inflammation and Antibody Responses in Multisystem Inflammatory Syndrome in Children (MIS-C). Cell. 2020 Nov 12;183(4):982-995.e14. doi: 10.1016/j.cell.2020.09.034. Epub 2020 Sep 14. PMID: 32991843; PMCID: PMC7489877.

17. Jeyanathan M, Afkhami S, Smaill F, Miller MS, Lichty BD, Xing Z. Immunological considerations for COVID-19 vaccine strategies. Nat Rev Immunol. 2020 Oct;20(10):615-632. doi: 10.1038/s41577-020-00434-6. Epub 2020 Sep 4. PMID: 32887954; PMCID: PMC7472682.

18. Escobar L.E, Molina-Cruz A, and Barillas-Mury C. BCG vaccine protection from severe coronavirus disease 2019 (COVID-19). *Proc Natl Acad Sci U S A* (2020) **117**(30): p. 17720-17726. doi: 10.1073/pnas.2008410117

19. Balasubramanian S, Rao NM, Goenka A, Roderick M, Ramanan AV. Coronavirus Disease 2019 (COVID-19) in Children - What We Know So Far and What We Do Not. Indian Pediatr. 2020 May 15;57(5):435-442. doi: 10.1007/s13312-020-1819-5. Epub 2020 Apr 9. PMID: 32273490; PMCID: PMC7240240.

20. Li H, Chen K, Liu M, Xu H, and Xu Q. The profile of peripheral blood lymphocyte subsets and serum cytokines in children with 2019 novel coronavirus pneumonia*. J Infect.* (2020) **81**(1): p. 115-120. doi: 10.1016/j.jinf.2020.04.001

21. Jacques FH, Apedaile E. Immunopathogenesis of COVID-19: Summary and Possible Interventions. Front Immunol. 2020 Sep 17;11:564925. doi: 10.3389/fimmu.2020.564925. PMID: 33072103; PMCID: PMC7533543.

22. Wu H, Zhu H, Yuan C, Yao C, Luo W, Shen X, et al. Clinical and Immune Features of Hospitalized Pediatric Patients With Coronavirus Disease 2019 (COVID-19) in Wuhan, China. *JAMA Netw Open*. (2020) **3**(6): p. e2010895. doi: 10.1001/jamanetworkopen.2020

23. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020 Mar 28;395(10229):1054-1062. doi: 10.1016/S0140-6736(20)30566-3. Epub 2020 Mar 11. Erratum in: Lancet. 2020 Mar 28;395(10229):1038. Erratum in: Lancet. 2020 Mar 28;395(10229):1038. PMID: 32171076; PMCID: PMC7270627.

24. Pierce C.A, Preston-Hurlburt P, Dai Y, Aschner C.B, Cheshenko N, Galen B, et al. Immune responses to SARS-CoV-2 infection in hospitalized pediatric and adult patients. *Sci Transl Med*. (2020) **12**(564). doi: 10.1126/scitranslmed.abd5487

25. Baumgarth N, Nikolich-Žugich J, Lee FE, Bhattacharya D. Antibody Responses to SARS-CoV-2: Let's Stick to Known Knowns. J Immunol. 2020 Nov 1;205(9):2342-2350. doi: 10.4049/jimmunol.2000839. Epub 2020 Sep 4. PMID: 32887754; PMCID: PMC7578055.

26. C. Rydyznski Moderbacher, Et al S. Crotty , Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. Cell **183**, 996–1012.e19 (2020).doi:10.1016/j.cell.2020.09.038pmid:33010815 CrossRefPubMedGoogle Scholar]

27. Gaebler C, Wang Z, Lorenzi JCC, Muecksch F, Finkin S, Tokuyama M, Cho A, Jankovic M, Schaefer-Babajew D, Oliveira TY, Cipolla M, Viant C, Barnes CO, Bram Y, Breton G, Hägglöf T, Mendoza P, Hurley A, Turroja M, Gordon K, Millard KG, Ramos V, Schmidt F, Weisblum Y, Jha D, Tankelevich M, Martinez-Delgado G, Yee J, Patel R, Dizon J, Unson-O'Brien C, Shimeliovich I, Robbiani DF, Zhao Z, Gazumyan A, Schwartz RE, Hatziioannou T, Bjorkman PJ, Mehandru S, Bieniasz PD, Caskey M, Nussenzweig MC. Evolution of antibody immunity to SARS-CoV-2. Nature. 2021 Jan 18. doi: 10.1038/s41586-021-03207-w. Epub ahead of print. PMID: 33461210.

28. Sallusto F, Lanzavecchia A, Araki K, Ahmed R. From vaccines to memory and back. Immunity. 2010 Oct 29;33(4):451-63. doi: 10.1016/j.immuni.2010.10.008. PMID: 21029957; PMCID: PMC3760154.

29. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell*. 2020;181(7):1489-1501.e15. doi:10.1016/j.cell.2020.05.015

30. Han H, Ma Q, Li C, Liu R, Zhao L, Wang W, et al. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. *Emerg Microbes Infect.* (2020) **9**(1): p. 1123-1130. doi: 10.1080/22221751.2020

31. Ramcharan T, Nolan O, Lai CY, Prabhu N, Krishnamurthy R, Richter AG, Jyothish D, Kanthimathinathan HK, Welch SB, Hackett S, Al-Abadi E, Scholefield BR, Chikermane A. Paediatric Inflammatory Multisystem Syndrome: Temporally Associated with SARS-CoV-2 (PIMS-TS): Cardiac Features, Management and Short-Term Outcomes at a UK Tertiary Paediatric Hospital. Pediatr Cardiol. 2020 Oct;41(7):1391-1401. doi: 10.1007/s00246-020-02391-2. Epub 2020 Jun 12. PMID: 32529358; PMCID: PMC7289638.

32. Yasuhara J, Kuno T, Takagi H, Sumitomo N. Clinical characteristics of COVID-19 in children: A systematic review. Pediatr Pulmonol. 2020 Oct;55(10):2565-2575. doi: 10.1002/ppul.24991. Epub 2020 Aug 4. PMID: 32725955.

33. Schuler BA, Habermann AC, Plosa EJ, Taylor CJ, Jetter C, Negretti NM, Kapp ME, Benjamin JT, Gulleman P, Nichols DS, Braunstein LZ, Hackett A, Koval M, Guttentag SH, Blackwell TS, Webber SA, Banovich NE; Vanderbilt COVID-19 Consortium Cohort; Human Cell Atlas Biological Network, Kropski JA, Sucre JM. Age-determined expression of priming protease TMPRSS2 and localization of SARS-CoV-2 in lung epithelium. J Clin Invest. 2021 Jan 4;131(1):e140766. doi: 10.1172/JCI140766. PMID: 33180746; PMCID: PMC7773394.

34. Lotfi M, Hamblin MR, Rezaei N. COVID-19: Transmission, prevention, and potential therapeutic opportunities. Clin Chim Acta. 2020 Sep;508:254-266. doi: 10.1016/j.cca.2020.05.044. Epub 2020 May 29. PMID: 32474009; PMCID: PMC7256510.

35. Lewis D. Why schools probably aren't COVID hotspots. Nature. 2020 Nov;587(7832):17. doi: 10.1038/d41586-020-02973-3. PMID: 33139909.

36. Rajmil L. Role of children in the transmission of the COVID-19 pandemic: a rapid scoping review. *BMJ Paediatr Open*. 2020;4(1):e000722. Published 2020 Jun 21. doi:10.1136/bmjpo-2020-000722

37. Kirby T. New variant of SARS-CoV-2 in UK causes surge of COVID-19 [published online ahead of print, 2021 Jan 5]. *Lancet Respir Med*. 2021;S2213-2600(21)00005-9. doi:10.1016/S2213-2600(21)00005-9

38. Tang JW, Toovey OTR, Harvey KN, Hui DDS. Introduction of the South African SARS-CoV-2 variant 501Y.V2 into the UK [published online ahead of

print, 2021 Jan 17]. *J Infect*. 2021;S0163-4453(21)00030-X. doi:10.1016/j.jinf.2021.01.007

39. Andreano E, Piccini G, Licastro D, Casalino L, Johnson NV, Paciello I, Monego SD, Pantano E, Manganaro N, Manenti A, Manna R, Casa E, Hyseni I, Benincasa L, Montomoli E, Amaro RE, McLellan JS, Rappuoli R. SARS-CoV-2 escape *in vitro* from a highly neutralizing COVID-19 convalescent plasma. bioRxiv [Preprint]. 2020 Dec 28:2020.12.28.424451. doi: 10.1101/2020.12.28.424451. PMID: 33398278; PMCID: PMC7781313.

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12th February 2021

To: Professor Imti Choonara,
Emeritus Professor in Child Health
Editor in Chief BMJ Paeds Open

Dear Imti,

Re: Understanding COVID-19; Are children the key?

Thank you so much for your encouraging email of the 10th of January.
I enclose the review as promised, on behalf of my co-authors.

We understand that it will go out for review as normal but look forward to hearing positive news shortly.

With very best wishes and stay safe

Deirdre Kelly CBE,
Professor of Paediatric Hepatology

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Understanding COVID-19; Are children the key?

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Understanding COVID-19; Are children the key?

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ABSTRACT

The devastating impact of the coronavirus 2019 (COVID-19) pandemic on global health and economic stability is immeasurable. The situation is dynamic and fast evolving with the world facing new variants of concern which may have immune escape potential. With threatened treatment and preventative strategies at stake, and the prospect of reinfection prolonging the pandemic, it is more crucial than ever to understand the pathogenesis of SARS-CoV-2 infection which intriguingly disproportionately affects adults and the elderly.

Children infected with SARS-CoV-2 remain largely asymptomatic or undergo a transient mild illness. Understanding why children have a milder phenotype and a significant survival advantage may help identify modifiable risk factors in adults. Current evidence suggests adults with COVID-19 show variability in innate and adaptive immune responses, which result in uncontrolled pro-inflammatory cytokine production in some patients, leading to severe disease and mortality. Children with acute COVID-19 infection seldom progress to acute respiratory distress syndrome (ARDS) and are less likely to exhibit the cytokine storm which is so prominent in adults. Even with the Kawasaki like illness, a hyperinflammation syndrome also known as paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 (PIMS-TS), mortality is low.

The key to successfully combating SARS-CoV-2 and future zoonotic pandemics may lie in understanding these critical differences and merits focussed consideration and research. The impact of community transmission amongst asymptomatic children is unknown; sustained global decline in infection rates and control of the COVID-19 pandemic may not be achieved until vaccination of children occurs.

In this review, we discuss the fundamental differences in the immune response between children and adults in the fight against SARS-CoV-2.

Key Statements:
Significant differences exist in the immune response to SARS-CoV-2 between children and adults; understanding the basis for this variance may be crucial to controlling the pandemic.
Emerging SARS-CoV-2 variants are more contagious and virulent. The role children have on community transmission of these new variants of concern needs to be established.
Sustained control of the pandemic may not be achieved until children are included in the global vaccination against SARS-CoV-2.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has had a catastrophic impact on world health and economic stability. It is hoped that social normalcy and economic restoration will return following global SARS-CoV-2 vaccination. The emergence of mutant strains raises concerns over the efficacy of recently licenced vaccines, questioning the degree of cross-immunity and protection afforded by these vaccines. Whether the programmes are successful or not, we must continue to decipher and understand the pathogenesis of this devastating infection which disproportionately affects adults and the elderly.

Children infected with SARS-CoV-2 are either asymptomatic or experience a milder phenotype compared to adults [1,2]. This trajectory is similar to that experienced in the SARS-CoV-1 and MERS epidemics of 2002 and 2012 [2]. COVID-19 symptoms of fever, cough and a loss of sense of smell or taste are less frequently encountered in children [1,2].

Pneumonitis progressing to acute respiratory distress syndrome (ARDS) and respiratory failure occurs in up to 19.5% of adult COVID-19 patients, with case fatality rates of 5-8% rising to 14.8% in elderly patients >80yrs [3,4]. Children are quoted to account for 1-5% of positive SARS-CoV-2 PCR tests but this is likely to be underrepresented as testing is skewed towards those who are symptomatic or require hospitalisation [5,6]. Of the children who test positive, severe illness accounts for 1-6%, with 65% of these developing the Kawasaki-like illness, paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 (PIMS-TS), also known as multisystem inflammatory syndrome in children (MIS-C), but even then, mortality is low [7].

Despite the milder phenotype, the pandemic has had a detrimental effect on child and adolescent health and education worldwide [8]. Healthcare access and delivery were disrupted with essential services like routine immunisations being postponed [8,9]. Children are also considered at low risk from COVID-19 and are therefore not a priority in vaccination trials.

A literature review was performed using the online database PubMed to summarise the available information on the differences and similarities in SARS-CoV-2 infection between children and adults, with focus on transmission, immune response and immunopathogenesis. Clinical syndromes will be referred to as adult COVID-19, paediatric COVID-19 and PIMS-TS.

TRANSMISSION

SARS-CoV-2 transmits via aerosol and droplet spread but the virus is also detected in stool samples [10]. Children experience more gastrointestinal symptoms of diarrhoea and vomiting than adults. Faecal-oral transmission nevertheless is not a common route of infection spread [10].

The role of children in SARS-CoV-2 transmission is considered small and reflective of levels of transmission in their community [11].

School and childcare centre COVID-19 outbreaks are not common especially if hand hygiene, wearing face masks (amongst teaching staff) and social distancing rules are followed [10,11], yet in the absence of testing asymptomatic individuals we are unable to track virus circulation in these settings. To date, contact tracing studies show 71-90% of childhood SARS-CoV-2 infections were identified following infection of a family member [11], so we are probably underestimating the proportion of asymptomatic infected children.

With the fall of the reproduction number (R) following the third national lockdown and success of the UK vaccination role out, schools reopened in March 2021 with pupils returning to face-to-face education. Cases of children testing positive for SARS-CoV-2 has since risen in the same way as observed following the reopening of schools in September 2020 [12]. These observations suggest that children and young adolescents are likely to transmit SARS-CoV-2 within the school setting, their household and the general population, strengthening the need to include them in vaccination programmes.

Clinical trials testing the efficacy of approved SARS-CoV-2 vaccines in children have commenced but programmes are currently paused until reports of rare clotting incidents in vaccinated young adults are fully investigated.

CHILDHOOD AND ADULT IMMUNE RESPONSES TO SARS-CoV-2

Host innate and adaptive immune interactions are paramount in the clearance of viral infections. Innate immune cells like circulating neutrophils and monocytes express pattern recognition receptors (PRR) e.g. Toll like receptors (TLR) which recognise 'danger signals' from microbial and endogenous molecules referred to as pathogen (PAMPs) and damage associated molecular patterns(DAMPs), respectively [13]. Innate cell activation occurs once these ligands bind to cell surface PPRs, leading to rapid pro-inflammatory cytokine production, immune cell recruitment, and resultant tissue inflammation. TLRs are present on antigen presenting cells (APCs) such as dendritic cells, monocytes and B lymphocytes. The Spike glycoprotein has a strong affinity to TLR4; TLR4 binding and activation is seen in SARS-CoV-1 and SARS-CoV-2 infection [14]. Moreover, APCs crucially link the innate and adaptive arms of the immune systems via the presentation of major histocompatibility complex proteins to induce T lymphocyte activation; MHC class I present antigen peptides to cytotoxic CD8 and MHC class II to helper CD4 T cells [15]. Cytotoxic CD8 cells kill virus infected cells with perforin and granzyme release in addition to other pro-inflammatory cytokines [13,15]. This T cell mediated immunity and humoral immunity forms the adaptive arm of the immune system and are discussed below in relation to SARS-CoV-2.

Adults with severe and critical COVID-19 display a dysregulated adaptive arm in combination with a hyperactivated innate response, leading to uncontrolled pro-inflammatory cytokine production, extensive immune-mediated lung injury and multi-organ failure [15,16].

Pneumonitis and ARDS are rarely seen in paediatric COVID-19 [16,17]. A hyper-inflammation element is however part of the immunophenotype observed in PIMS-TS, a condition which characteristically occurs in children who are SARS-CoV-2 PCR negative and seropositive [18].

1. Innate Immunity

A lack of control of the primary SARS-CoV-2 infection and fatal COVID-19 has been strongly associated with hyperacute but ineffective innate immunity in adults [15,16].

High numbers of circulating monocytes and neutrophils are found in severe adult COVID-19. Classical (CD14+ CD16-) and intermediate monocytes (CD14++ CD16+), identified by fluorescence-activated cell sorting analysis (FACS), produce the inflammatory cytokines seen in severe COVID-19 [16]. These activated innate cells are rarely found in asymptomatic individuals or those with mild disease. Interestingly, non-classical monocytes (CD14+ CD16++), which are anti-inflammatory and have a role in maintaining endothelial integrity, are reduced in number in severe adult COVID-19 (Figure 1) [16,17].

Aside from phagocytosis, activated neutrophils form neutrophil extracellular traps (NETS) which are aptly named net-like structures made up mostly of chromatin and extracellular proteins that 'trap' microbes and infected cells. These NETS can prime the clotting cascade. NETS have thus been implicated in the microangiopathy and thrombosis seen in COVID-19 [17]. Children with PIMS-TS have endothelial cell damage and micro-thrombosis, especially seen in their skin lesions [18]. The underlying microvascular and thrombotic pathogenesis may be shared in severe adult COVID-19 and PIMS-TS.

Natural Killer (NK) cells are of lymphocyte lineage, however, they are considered part of the innate immune system with their rapid ability to kill virus infected cells by perforin and granzyme degranulation. NK cells are consistently reduced in COVID-19 but this is most marked in severe disease [16]. Lower numbers of perforin producing NK cells were found in intensive care unit (ICU) COVID-19 patients compared to non-ICU patients in one study [19]. This implies that NK cells are not only reduced in number but are also functionally impaired in severe disease. NK cell numbers are preserved in paediatric COVID-19. FACS analysis of peripheral blood show similar reductions in NK cells and non-classical monocytes in children with PIMS-TS [20].

Another theory for why children are more resilient to COVID-19 is trained immunity, the functional reprogramming of innate immune cells following common childhood infections and vaccinations, enabling memory cell formation which was previously considered unique to the adaptive immune system [1,2,6]. This heterogeneous immunity is described with the Bacillus Calmette–Guérin (BCG) vaccination [21] and is speculated to occur with co-infections; 10% of co-infections occur in children testing positive for SARS-CoV-2 [21].

APCs like dendritic cells (DCs) are suppressed in SARS-CoV-2 which indirectly downregulates T cell activation and function [17]. DCs produce interferon (IFN) I and III which are powerful anti-viral cytokines important in early viral responses. SARS-CoV-2 like SARS-CoV-1 and MERS, are capable of immune escape by IFN inhibition [17], thereby eluding one of the hosts most effective anti-viral measures. One study found 10% of adults with life-threatening COVID-19 had autoantibodies directed against IFN I, reinforcing the significance of IFN and the detrimental effect of select immune deficiencies [22].

2. Adaptive immunity

a. Lymphopenia & lymphocyte exhaustion

The lymphopenia and high neutrophil-to-lymphocyte ratio described in severe adult COVID-19 is rarely observed in children [23,24]. In adults, CD4+ and CD8+ naïve and memory T cell numbers are reduced [25]. Conversely, lymphocyte counts are preserved in paediatric COVID-19 but reduced in PIMS-TS [6,20].

Adult patients with mild COVID-19 have clonally expanded CD8+ T lymphocytes [25]. Moreover, individuals with mild disease were shown to mount a stronger SARS-CoV-2 specific T cell response compared to those with severe disease, implying preserved adaptive immunity corresponds with superior disease control and recovery (Figure 2) [17,25].

In addition, T lymphocytes from severe COVID-19 patients have higher expression of the cell exhaustion and inhibition markers PD-1, LAG-3 and TIM3 [24]. Compared to healthy controls, CD8+ T lymphocytes and NK cells in adult COVID-19 also have higher surface expression of the NKG2A inhibitory receptor; levels return to normal with recovery [17,24]. T cell exhaustion correlates with impaired effector function. These findings suggest cellular immunity is affected both quantitatively and functionally.

T cell function and survival is a potential novel area of research in children with COVID-19; findings will contribute to our understanding of the age-related T cell response and behaviour in COVID-19.

b. Humoral response

Adult COVID-19 patients mount a strong B cell response; neutralizing antibodies against the Spike glycoprotein are detected in the majority of convalescent COVID-19 patients [17]. Although total B cells numbers are reduced, plasma cell levels are paradoxically increased (Figure 3) [16]. IgG and IgA levels are transiently increased in adult asymptomatic patients and those with mild disease [16]. Adults with severe COVID-19 have persistently raised IgG and IgA levels. This may correspond with higher neutralising antibodies but higher IgG and IgA levels may also perpetuate innate cell activity, primarily in neutrophils expressing surface Fc receptors [26]. Early phase rise in IgM levels were seen in asymptomatic, mild and severe cases [16]. Early phase rise in IgM, persistent IgG and waning IgA levels were observed in paediatric COVID-19 [16,17].

In contrast, no IgM was detected in children with PIM-TS [27]. These children were also SARS-CoV-2PCR negative, signifying that PIMS-TS is a clinical syndrome which occurs weeks following the acute infection [27]. Children with PIMS-TS maintained high IgG levels with enduring Fc receptor-binding capacity which were capable of monocyte activation and sustained hyper-inflammatory activity [26].

Lastly, a study in hospitalised adults and children with COVID-19 in New York added to the cumulative evidence that mild disease seen in children was unlikely owed solely to adaptive immune responses; adults with severe COVID-19 mounted strong T cell responses and had higher neutralizing antibodies compared to children with COVID-19 and/or PIMS-TS [28].

c. Anamnestic responses

The course of severe adult COVID-19 is relatively slow (median 19 days post-symptom onset for fatal cases [28,29]), suggesting the protective involvement of memory B cells and T cells take days to develop [29]. Virus-specific memory CD4+ and CD8+ T cell responses persist for at least six months, whereas virus-specific antibody responses declined after three months of infection [30]. Moreover, SARS-CoV-2-specific memory B cell responses evolved between 1.3 and 6.2 months after infection in a manner that was consistent with antigen persistence; viral antigens were detectable in enterocytes from adult intestinal biopsies even months after infection [31].

Immune responses shortly after resolution of infection are not predictive of long-term memory in adults [31]. Learning from SARS-CoV-1 and MERS, it is likely that few antibodies may persist for 2-3 years after infection but T cell memory responses may last longer. Indeed, 28-50% of adults not previously exposed to SARS-CoV-2 infection had cross-reactive T cells, likely mounted against other members of the coronavirus family that includes the common cold coronaviruses HCoV-OC43, HCoV-HKU1, HCoV-229E and HCoV-NL63 [32]. Children may have cross-protective antibodies against SARS-CoV-2 from the seasonal coronaviruses which commonly infect children [1,2,6].

Immunosenescence, the natural decline in innate and adaptive immune cell function occurring with age is most pronounced in elderly patients >80yrs old [2,6,17]. Immunosenescence may therefore predispose patients to poor viral clearance, promoting COVID-19 progression.

3. Cytokine storm

The hyperinflammatory response known as 'cytokine storm' occurs in the second week in severe and life-threatening adult COVID-19; higher circulating pro-inflammatory cytokines are observed and clinically patients develop ARDS, multiorgan failure and DIC [3,4]. In ARDS, the cytokine storm is preceded by pulmonary infiltration of macrophages and neutrophils [3,4]. These innate cells secrete powerful inflammatory cytokines including interleukin-6 (IL-6), IL-12, IL-10, IFN-γ and TNF-α and with varying reports of raised IL-1β, IL-7, IL-8, IL-17 and G-CSF [21,33]. Levels of IL-6, IL-10 along with C-reactive protein (CRP) are considered prognostic markers (Table 1) [21,33]. Lymphopenia, raised ferritin and D-Dimer levels also correspond with disease severity in adult COVID-19 [4].

These clinical manifestations are rare in paediatric COVID-19; of children who develop severe illness, 2/3 will be due to PIMS-TS [7]. PIMS-TS presents with a wide array of clinical features and variable disease severity with shock, multi-organ failure, left ventricular impairment and coronary artery abnormalities described amongst them [34]. The underlying cytokine storm in PIMS-TS is referred to as the

macrophage activation syndrome (MAS) [27,34]. MAS is encountered infrequently with other childhood infections e.g. Epstein-Barr virus (EBV), with medications and as a complication of autoimmune diseases [6,15,17].

Pierce and colleagues observed PIMS-TS patients to have higher IL-6, IFN-γ, TNF-α and CXCL10 compared to acute paediatric COVID-19 [28]. The chemokines CXCL10 (aka IP-10), CXCL8, CCL2 (aka MCP-1) are likewise detected in large amounts in adult COVID-19 (Table 2) [33]. Other studies found levels of IL-6, IFN-γ and TNF-α to be unchanged in paediatric COVID-19 [23].

In terms of laboratory biomarkers, PIMS-TS patients, similar to severe adult COVID-19, have higher CRP, ferritin and D-Dimer levels, reflecting the inflammatory nature of the underlying pathology [7].

These findings highlight the striking parallels in the immune responses and the biomarker levels observed between severe adult COVID-19 and PIMS-TS. The resulting end organ damage is however, very different for these hyper-inflammatory conditions.

PIMS-TS does not appear to be a direct result of SARS-CoV-2 infection but occurs following host immune attenuation after SARS-CoV-2 exposure [17]. This concept is supported by the observation that PIMS-TS cases arose weeks following the peak of the 1st wave of COVID-19 and most children were SARS-CoV-2 PCR negative and IgG positive at the time of presentation [27,34]. In contrast, adult COVID-19 patients may remain PCR-positive at the time of disease progression.

4. ACE2 expression

The expression of the SARS-CoV-2 entry receptor, Angiotensin-converting enzyme 2 (ACE2) has an almost Gaussian distribution, increasing from childhood into adulthood and declining with old age [1,2,5]. It is found in most tissues but is highly expressed on type 2 pneumocytes [1,2]. Evidence points towards a protective role but the exact link between ACE2 and COVID-19 disease severity is unclear. ACE2 downregulation is seen in adults with comorbidities like hypertension and diabetes and also upon SARS-CoV-2 Spike protein binding [1,2]. Animal studies suggests ACE2 protects against lung injury induced by SARS-CoV-2 [2,5].

Affinity for ACE2 may influence disease severity and trend; ACE2 in children are speculated to have a lower affinity to SARS-CoV-2 thus preventing viral entry into the host cell [5].

TMPRSS2, transmembrane serine protease 2, helps to prime the Spike glycoprotein and promotes viral entry [35]. Lower TMPRSS2 levels are found in children and infants. These differences in ACE2 and TMPRSS2 expression may be pivotal in helping us understand the discrepancies in the clinical outcomes between children and adults.

VIROLOGY AND VACCINES

Since the announcement in December 2020 of a new COVID-19 strain in the UK, N501Y, multiple variants of concern have been identified. This includes the E484K mutation first detected in South Africa, the P1.variant from Brazil and the double variant with both E484Q and L452R mutations identified in India [36,37]. Worryingly these new strains are not only more infectious but are more virulent, causing greater morbidity and mortality [36,37]. There is no evidence to-date to establish children's susceptibility to the new variants, yet school closures in January 2021 in the UK were implemented to curb transmission of the more infectious strains.

With the SARS-CoV-2 vaccination programmes underway, there are serious concerns that mutations in the Spike glycoprotein may render vaccine-induced antibodies ineffective or sub-neutralising [38]. The current COVID-19 vaccines are still considered to be effective against the N501Y variant but may be less so against the other new variants of concern [37,38].

Due to the global push to prevent mortality in high-risk groups, children are a low priority group for vaccination. Currently, the UK Joint Committee on Vaccination and Immunisation (JCVI) advises that only children from high risk groups such as those with severe neurological disabilities who require residential care will be considered for vaccination.
(https://www.gov.uk/government/publications/priority-groups-for-coronavirus-covid-19-vaccination-advice-from-the-jcvi-30-december-2020).

The contribution children have on viral transmission and disease perpetuation in the community is uncertain. A rise in cases following the reopening of schools suggests the impact asymptomatic children have on transmission rates may be underestimated. This supports the argument that children are an important group to vaccinate. The United States saw a large reduction in rates of invasive pneumococcal disease in all ages caused by serotypes included in the pneumococcal vaccines PCV7 and PCV13 after routine use of these vaccines began for children (https://www.cdc.gov/vaccines/vpd/pneumo/public/index.html). Sustained global decline in infection rates and control of the COVID-19 pandemic may not be achieved until children are included in the vaccination programmes.

SUMMARY

Striking clinical and immune differences exist between children and adult COVID-19. Children are largely asymptomatic and the dysregulated innate response is rarely described in paediatric COVID-19.

Conversely, severe adult COVID-19 and PIMS-TS appear to share similar immune signatures yet their clinical response and end organ damage are distinctly different.

The impact children have on viral transmission and disease perpetuation is uncertain; with the rise of cases in schools, including children in vaccination programmes will help curb the spread and control the pandemic.

CONCLUSION

The COVID-19 pandemic has highlighted gaps in our knowledge of viral immunology between adults and children. Deciphering the mechanism of children's resilience to disease could help target therapeutic interventions in adults and should be of high priority for future investigations.

REFERENCES

1. Miri SM, Noorbakhsh F, Mohebbi SR, Ghaemi A. Higher prevalence of asymptomatic or mild COVID-19 in children, claims and clues. J Med Virol. 2020 Nov;92(11):2257-2259. doi: 10.1002/jmv.26069. Epub 2020 Jun 9. PMID: 32470174; PMCID: PMC7283682.

2. Dhochak N, Singhal T, Kabra SK, Lodha R. Pathophysiology of COVID-19: Why Children Fare Better than Adults? Indian J Pediatr. 2020 Jul;87(7):537-546. doi: 10.1007/s12098-020-03322-y. Epub 2020 May 14. PMID: 32410003; PMCID: PMC7221011.

3. Zhu J, Ji P, Pang J, Zhong Z, Li H, He C, Zhang J, Zhao C. Clinical characteristics of 3062 COVID-19 patients: A meta-analysis. J Med Virol. 2020 Oct;92(10):1902-1914. doi: 10.1002/jmv.25884. Epub 2020 Jun 24. PMID: 32293716; PMCID: PMC7262119.

4. Nanda A, Vura NVRK, Gravenstein S. COVID-19 in older adults. Aging Clin Exp Res. 2020 Jul;32(7):1199-1202. doi: 10.1007/s40520-020-01581-5. Epub 2020 May 10. PMID: 32390064; PMCID: PMC7211267.

5. Zimmermann P, Curtis N. Why is COVID-19 less severe in children? A review of the proposed mechanisms underlying the age-related difference in severity of SARS-CoV-2 infections. Arch Dis Child. 2020 Dec 1:archdischild-2020-320338. doi: 10.1136/archdischild-2020-320338. Epub ahead of print. PMID: 33262177.

6. Felsenstein S, Hedrich CM. SARS-CoV-2 infections in children and young people. Clin Immunol. 2020 Nov;220:108588. doi: 10.1016/j.clim.2020.108588. Epub 2020 Sep 6. PMID: 32905851; PMCID: PMC7474910.

7. Yasuhara J, Kuno T, Takagi H, Sumitomo N. Clinical characteristics of COVID-19 in children: A systematic review. Pediatr Pulmonol. 2020

Oct;55(10):2565-2575. doi: 10.1002/ppul.24991. Epub 2020 Aug 4. PMID: 32725955.

8. Araújo LA, Veloso CF, Souza MC, Azevedo JMC, Tarro G. The potential impact of the COVID-19 pandemic on child growth and development: a systematic review [published online ahead of print, 2020 Sep 23]. *J Pediatr (Rio J)*. 2020;S0021-7557(20)30209-6. doi:10.1016/j.jped.2020.08.008

9. Ashikkali L, Carroll W, Johnson C. The indirect impact of COVID-19 on child health. *Paediatr Child Health (Oxford)*. 2020;30(12):430-437. doi:10.1016/j.paed.2020.09.004

10. Lotfi M, Hamblin MR, Rezaei N. COVID-19: Transmission, prevention, and potential therapeutic opportunities. Clin Chim Acta. 2020 Sep;508:254-266. doi: 10.1016/j.cca.2020.05.044. Epub 2020 May 29. PMID: 32474009; PMCID: PMC7256510.

11. Rajmil L. Role of children in the transmission of the COVID-19 pandemic: a rapid scoping review. *BMJ Paediatr Open*. 2020;4(1):e000722. Published 2020 Jun 21. doi:10.1136/bmjpo-2020-000722

12. Bhuiyan MU, Stiboy E, Hassan MZ, Chan M, Islam MS, Haider N, Jaffe A, Homaira N. Epidemiology of COVID-19 infection in young children under five years: A systematic review and meta-analysis. Vaccine. 2021 Jan 22;39(4):667-677. doi: 10.1016/j.vaccine.2020.11.078. Epub 2020 Dec 5. PMID: 33342635; PMCID: PMC7833125.

13. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. Nat Immunol. 2015 Apr;16(4):343-53. doi: 10.1038/ni.3123. PMID: 25789684; PMCID: PMC4507498.

14. Aboudounya MM, Heads RJ. COVID-19 and Toll-Like Receptor 4 (TLR4): SARS-CoV-2 May Bind and Activate TLR4 to Increase ACE2 Expression, Facilitating

Entry and Causing Hyperinflammation. Mediators Inflamm. 2021 Jan 14;2021:8874339. doi: 10.1155/2021/8874339. PMID: 33505220; PMCID: PMC7811571.

15. Wieczorek M, Abualrous ET, Sticht J, et al. Major Histocompatibility Complex (MHC) Class I and MHC Class II Proteins: Conformational Plasticity in Antigen Presentation. *Front Immunol*. 2017;8:292. Published 2017 Mar 17. doi:10.3389/fimmu.2017.00292

16. Carsetti R, Zaffina S, Piano Mortari E, Terreri S, Corrente F, Capponi C, Palomba P, et al. Different Innate and Adaptive Immune Responses to SARS-CoV-2 Infection of Asymptomatic, Mild, and Severe Cases. Front Immunol. 2020 Dec 16;11:610300. doi: 10.3389/fimmu.2020.610300. PMID: 33391280; PMCID: PMC7772470.

17. Bordallo B, Bellas M, Cortez AF, Vieira M, Pinheiro M. Severe COVID-19: what have we learned with the immunopathogenesis? Adv Rheumatol. 2020 Sep 22;60(1):50. doi: 10.1186/s42358-020-00151-7. PMID: 32962761; PMCID: PMC7506814.

18. Gkoutzourelas A, Bogdanos DP, Sakkas LI. Kawasaki Disease and COVID-19. *Mediterr J Rheumatol*. 2020;31(Suppl 2):268-274. Published 2020 Sep 21. doi:10.31138/mjr.31.3.268

19. Bordoni V, Sacchi A, Cimini E, Notari S, Grassi G, Tartaglia E, Casetti R, Giancola ML, Bevilacqua N, Maeurer M, Zumla A, Locatelli F, De Benedetti F, Palmieri F, Marchioni L, Capobianchi MR, D'Offizi G, Petrosillo N, Antinori A, Nicastri E, Ippolito G, Agrati C. An Inflammatory Profile Correlates With Decreased Frequency of Cytotoxic Cells in Coronavirus Disease 2019. Clin Infect Dis. 2020 Nov 19;71(16):2272-2275. doi: 10.1093/cid/ciaa577. PMID: 32407466; PMCID: PMC7239202.

20. Gruber CN, Patel RS, Trachtman R, Lepow L, Amanat F, Krammer F, Wilson KM, Onel K, Geanon D, Tuballes K, Patel M, Mouskas K, O'Donnell T, Merritt E, Simons NW, Barcessat V, Del Valle DM, Udondem S, Kang G, Gangadharan S, Ofori-Amanfo G, Laserson U, Rahman A, Kim-Schulze S, Charney AW, Gnjatic S, Gelb BD, Merad M, Bogunovic D. Mapping Systemic Inflammation and Antibody Responses in Multisystem Inflammatory Syndrome in Children (MIS-C). Cell. 2020 Nov 12;183(4):982-995.e14. doi: 10.1016/j.cell.2020.09.034. Epub 2020 Sep 14. PMID: 32991843; PMCID: PMC7489877.

21. Balasubramanian S, Rao NM, Goenka A, Roderick M, Ramanan AV. Coronavirus Disease 2019 (COVID-19) in Children - What We Know So Far and What We Do Not. Indian Pediatr. 2020 May 15;57(5):435-442. doi: 10.1007/s13312-020-1819-5. Epub 2020 Apr 9. PMID: 32273490; PMCID: PMC7240240.

22. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann HH, Zhang Y, et a;/ NIAID-USUHS Immune Response to COVID Group; COVID Clinicians; COVID-STORM Clinicians; Imagine COVID Group; French COVID Cohort Study Group; Milieu Intérieur Consortium; CoV-Contact Cohort; Amsterdam UMC Covid-19 Biobank; COVID Human Genetic Effort, Tsang JS, Goldbach-Mansky R, Kisand K, Lionakis MS, Puel A, Zhang SY, Holland SM, Gorochov G, Jouanguy E, Rice CM, Cobat A, Notarangelo LD, Abel L, Su HC, Casanova JL. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science. 2020 Oct 23;370(6515):eabd4585. doi: 10.1126/science.abd4585. Epub 2020 Sep 24. PMID: 32972996; PMCID: PMC7857397.

23. Li H, Chen K, Liu M, Xu H, and Xu Q. The profile of peripheral blood lymphocyte subsets and serum cytokines in children with 2019 novel coronavirus pneumonia. *J Infect.* (2020) **81**(1): p. 115-120. doi: 10.1016/j.jinf.2020.04.001

24. Jacques FH, Apedaile E. Immunopathogenesis of COVID-19: Summary and Possible Interventions. Front Immunol. 2020 Sep 17;11:564925. doi: 10.3389/fimmu.2020.564925. PMID: 33072103; PMCID: PMC7533543.

25. Wu H, Zhu H, Yuan C, Yao C, Luo W, Shen X, et al. Clinical and Immune Features of Hospitalized Pediatric Patients With Coronavirus Disease 2019 (COVID-19) in Wuhan, China. *JAMA Netw Open*. (2020) **3**(6): p. e2010895. doi: 10.1001/jamanetworkopen.2020

26. Bartsch YC, Wang C, Zohar T, Fischinger S, Atyeo C, Burke JS, Kang J et al. Humoral signatures of protective and pathological SARS-CoV-2 infection in children. Nat Med. 2021 Mar;27(3):454-462. doi: 10.1038/s41591-021-01263-3. Epub 2021 Feb 12. PMID: 33589825.

27. Perez-Toledo M, Faustini SE, Jossi SE, Shields AM, Kanthimathinathan HK, Allen JD, Watanabe Y, Goodall M, Wraith DC, Veenith TV, Drayson MT, Jyothish D, Al-Abadi E, Chikermane A, Welch SB, Masilamani K, Hackett S, Crispin M, Scholefield BR, Cunningham AF, Richter AG. Serology confirms SARS-CoV-2 infection in PCR-negative children presenting with Paediatric Inflammatory Multi-System Syndrome. medRxiv [Preprint]. 2020 Jun 7:2020.06.05.20123117. doi: 10.1101/2020.06.05.20123117. PMID: 32577677; PMCID: PMC7302282.

28. Pierce C.A, Preston-Hurlburt P, Dai Y, Aschner C.B, Cheshenko N, Galen B, et al. Immune responses to SARS-CoV-2 infection in hospitalized pediatric and adult patients. *Sci Transl Med*. (2020) **12**(564). doi: 10.1126/scitranslmed.abd5487

29. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020 Mar 28;395(10229):1054-1062. doi: 10.1016/S0140-6736(20)30566-3. Epub 2020

Mar 11. Erratum in: Lancet. 2020 Mar 28;395(10229):1038. Erratum in: Lancet. 2020 Mar 28;395(10229):1038. PMID: 32171076; PMCID: PMC7270627.

30. Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. Cell. 2021 Feb 18;184(4):861-880. doi: 10.1016/j.cell.2021.01.007. Epub 2021 Jan 12. PMID: 33497610; PMCID: PMC7803150.

31. Gaebler C, Wang Z, Lorenzi JCC, Muecksch F, Finkin S, Tokuyama M, Cho A, Jankovic M, Schaefer-Babajew D, Oliveira TY, Cipolla M, Viant C, Barnes CO, Bram Y, Breton G, Hägglöf T, Mendoza P, Hurley A, Turroja M, Gordon K, Millard KG, Ramos V, Schmidt F, Weisblum Y, Jha D, Tankelevich M, Martinez-Delgado G, Yee J, Patel R, Dizon J, Unson-O'Brien C, Shimeliovich I, Robbiani DF, Zhao Z, Gazumyan A, Schwartz RE, Hatziioannou T, Bjorkman PJ, Mehandru S, Bieniasz PD, Caskey M, Nussenzweig MC. Evolution of antibody immunity to SARS-CoV-2. Nature. 2021 Jan 18. doi: 10.1038/s41586-021-03207-w. Epub ahead of print. PMID: 33461210.

32. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell*. 2020;181(7):1489-1501.e15. doi:10.1016/j.cell.2020.05.015

33. Han H, Ma Q, Li C, Liu R, Zhao L, Wang W, et al. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. *Emerg Microbes Infect.* (2020) **9**(1): p. 1123-1130. doi: 10.1080/22221751.2020

34. Ramcharan T, Nolan O, Lai CY, Prabhu N, Krishnamurthy R, Richter AG, Jyothish D, Kanthimathinathan HK, Welch SB, Hackett S, Al-Abadi E, Scholefield BR, Chikermane A. Paediatric Inflammatory Multisystem Syndrome: Temporally Associated with SARS-CoV-2 (PIMS-TS): Cardiac Features, Management and Short-Term Outcomes at a UK Tertiary Paediatric

Hospital. Pediatr Cardiol. 2020 Oct;41(7):1391-1401. doi: 10.1007/s00246-020-02391-2. Epub 2020 Jun 12. PMID: 32529358; PMCID: PMC7289638

35. Schuler BA, Habermann AC, Plosa EJ, Taylor CJ, Jetter C, Negretti NM, Kapp ME, Benjamin JT, Gulleman P, Nichols DS, Braunstein LZ, Hackett A, Koval M, Guttentag SH, Blackwell TS, Webber SA, Banovich NE; Vanderbilt COVID-19 Consortium Cohort; Human Cell Atlas Biological Network, Kropski JA, Sucre JM. Age-determined expression of priming protease TMPRSS2 and localization of SARS-CoV-2 in lung epithelium. J Clin Invest. 2021 Jan 4;131(1):e140766. doi: 10.1172/JCI140766. PMID: 33180746; PMCID: PMC7773394.

36. Faria NR, Mellan TA, Whittaker C, et al. Genomics and epidemiology of a novel SARS-CoV-2 lineage in Manaus, Brazil. Preprint. *medRxiv*. 2021;2021.02.26.21252554. Published 2021 Mar 3. doi:10.1101/2021.02.26.21252554

37. Tang JW, Toovey OTR, Harvey KN, Hui DDS. Introduction of the South African SARS-CoV-2 variant 501Y.V2 into the UK [published online ahead of print, 2021 Jan 17]. *J Infect*. 2021;S0163-4453(21)00030-X. doi:10.1016/j.jinf.2021.01.007

38. Andreano E, Piccini G, Licastro D, Casalino L, Johnson NV, Paciello I, Monego SD, Pantano E, Manganaro N, Manenti A, Manna R, Casa E, Hyseni I, Benincasa L, Montomoli E, Amaro RE, McLellan JS, Rappuoli R. SARS-CoV-2 escape *in vitro* from a highly neutralizing COVID-19 convalescent plasma. bioRxiv [Preprint]. 2020 Dec 28:2020.12.28.424451. doi: 10.1101/2020.12.28.424451. PMID: 33398278; PMCID: PMC7781313.

Figure 1. Innate cell frequencies in SARS-CoV-2 infection

NK cells, Natural killer cells; PIMS-TS, paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2; ↑, increased; ↓, decreased; ↔, unchanged. Ref 15, 16, 17, 18.

Figure 2. T lymphocyte populations in SARS-CoV-2 infection

CD, cluster of differentiation; PIMS-TS, paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2; ↑, increased; ↓, decreased; ↔, unchanged. Ref 16, 19, 22, 23, 24.

Figure 3. B lymphocyte populations in SARS-CoV-2 infection

IgG, immunoglobulin G; IgA, immunoglobulin A; PIMS-TS, paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2; ↑, increased; ↓, decreased; ↔, unchanged. Ref 15, 16, 25, 26.

Table 1. Cytokine production in children and adults with SARS-CoV-2 infection

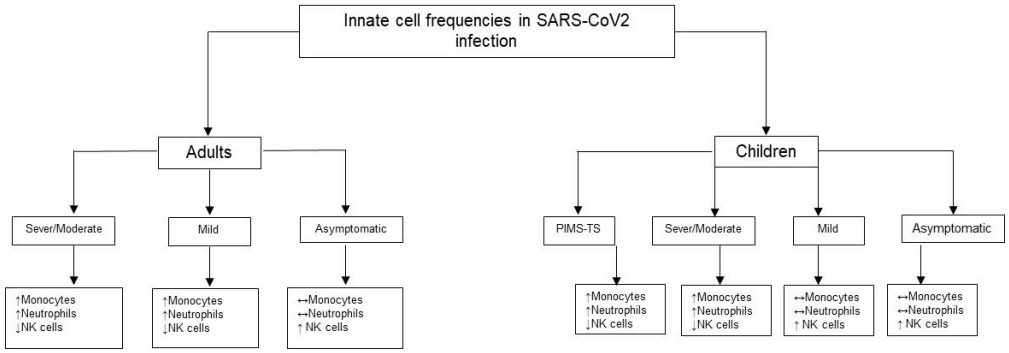
	ADULTS			CHILDREN			
	Severe	**Mild**	**Asymptomatic**	**PIMS-TS**	**Severe**	**Mild**	**Asymptomatic**
CYTOKINES	↑IL-6	↑IL-6	↔IL-6	↑IL-6	↔IL-6	↔IL-6	↔IL-6
	↑ IL-10	↑IL-10	↑ IL-10	↑ IL-10	↑ IL-10	↑ IL-10	↑ IL-10
	↑IFN	↑ IFN	↔IFN	↑IFN	↑/↔IFN	↑/↔IFN	↔IFN
	↑ TNFα	↑ TNFα	↔ TNFα	↑ TNFα	↔ TNFα	↔ TNFα	↔ TNFα
	↑/↔ IL-1β	↑/↔ IL-1β	↔ IL-1β	n/a	↔ IL-1β	↔ IL-1β	↔ IL-1β
	↑/↔ IL-8	↑/↔ IL-8	↔ IL-8	↑ IL-8	↑ IL-8	↔ IL-8	↔ IL-8
	↑/↔ IL-17	↑/↔ IL-17	↔ IL-17	↑ IL-17	↑/↔ IL-17	↑/↔ IL-17	↔ IL-17

n/a, not available; ↑, increased; ↓, decreased; ↔, unchanged; interleukin; IFN, Interferon; TNFα, Tumour necrosis factor alpha; PIMS-TS, paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2. Ref 20, 26, 32, 33.

Table 2. Chemokine receptor expression in children and adults with SARS-CoV-2 infection

	ADULTS			CHILDREN			
	Severe	**Mild**	**Asymptomatic**	**PIMS-TS**	**Severe**	**Mild**	**Asymptomatic**
CHEMOKINE RECEPTORS	↑CXCL10	↑CXCL10	↔CXCL10	↑CXCL10	↔CXCL10	↔CXCL10	↔CXCL10
	↑CXCL8	↑CXCL8	↔CXCL8	n/a	↔CXCL8	↔CXCL8	↔CXCL8
	↑CCL2	↑CCL2	↔CCL2	↔CCL2	↔CCL2	↔CCL2	↔CCL2

n/a, not available; ↑, increased; ↓, decreased; ↔, unchanged; CXCL-, chemokine (C-X-C motif) ligand; CCL-, CC chemokine ligand; PIMS-TS, paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2.
Ref 22, 27, 32.



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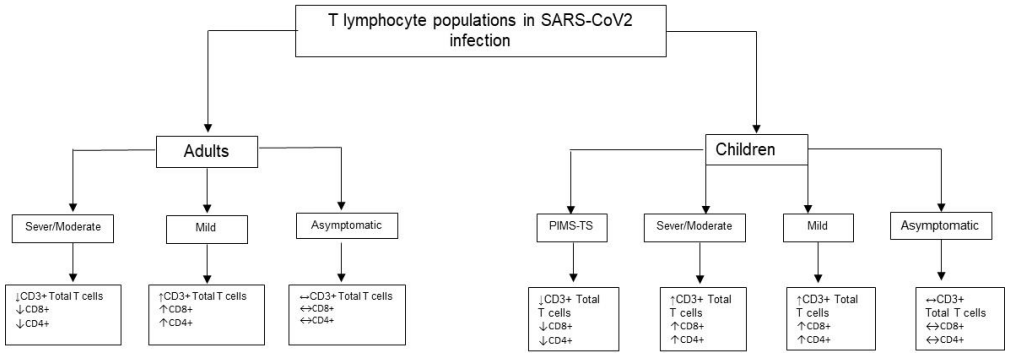
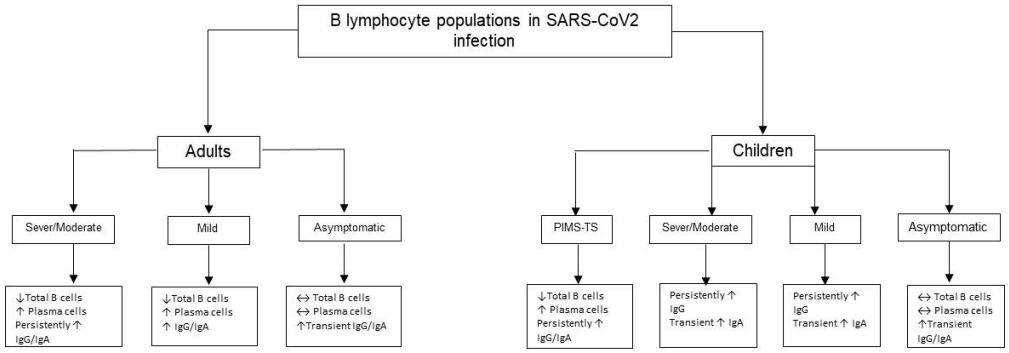


250x87mm (120 x 120 DPI)



248x86mm (120 x 120 DPI)